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14. ABSTRACT

Short CDP/Cux isoforms were found to be overexpressed in breast cancer cell lines, in human breast tumors and in uterine leiomyomas, suggesting that these proteins play a key role in tumor development and progression. My project consists in analyzing the effect of these CDP/Cux isoforms on mammary gland development and tumorigenesis. Also, I will work on the identification of targets of CDP/Cux that mediate its oncogenic properties. So far, I have shown that overexpressing short CDP/Cux isoforms lead to abnormal development of the mammary gland. Furthermore, overexpressing p75, p110 or p200 CDP/Cux leads to the development of mammary gland tumors in mice. These tumors seem to be of basal origin, suggesting that CDP/Cux promotes tumorigenesis in a precursor cell. Breast tumor patients with similar types of disease have very low chances of survival, since no specific treatment is currently available for them. Thus, my research project will enable us to gain a better understanding of the biological functions of each CDP/Cux isoform in mammary gland development and tumorigenesis, which could possibly lead to new therapeutic targets for the treatment of basal breast cancers.

15. SUBJECT TERMS
Cancer, Breast Cancer, Oncogene, Proliferation, CDP/Cux, Transcription factor

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INTRODUCTION

The CDP/Cux transcription factor is an important regulator of cell cycle progression, and was also found to be involved in many other processes, such as determination of cell-type identity, cell growth control, as well as cell migration and invasion (*1-4*). In addition, short CDP/Cux isoforms were found to be overexpressed in breast cancer cell lines, in human breast tumors and in uterine leiomyomas, suggesting that these proteins could play a key role in tumor development and progression (*5, 6*). I have also previously shown that overexpression of p75 CDP/Cux results in the development of myeloproliferative disease-like myeloid leukemia in mice (*7*). My project consists in analyzing the effects of these CDP/Cux isoforms on mammary gland development and tumorigenesis, which will allow us to demonstrate the oncogenic role of CDP/Cux on mammary epithelial cells. I will also work on the identification of targets of CDP/Cux, the deregulation of which could lead to cancer progression. My research project will enable us to gain a better understanding of the biological functions of each CDP/Cux isoform in mammary gland development and tumorigenesis. Any knowledge gained in this area would be very important, as it could lead to new therapeutic targets for the treatment of breast cancer. Transgenic mice overexpressing p75, p110 or p200 CDP/Cux under the control of the mouse mammary tumor virus promoter (MMTV) specifically integrated into the hprt locus were generated. These mice were found to display anomalies in mammary gland development and to develop tumors, which is what I will describe in this report.

BODY

1-Determine the effect of the overexpression of the CDP/Cux p200, p110 and p75 isoforms on mammary gland development (Task 1)

Transgenic Construct and Expression

To determine the role of the CDP/Cux transcription factor on mammary gland tumorigenesis, transgenic mice overexpressing one of the three CDP/Cux isoforms, p200, p110 and p75, were generated. The transgene was integrated by specific transgenesis into the Hprt locus under the control of the MMTV-LTR promoter (Appendix 1A) (*8*). These lines were originally of mixed background and were backcrossed for seven generations towards the C57BL/6 and the FVB/N backgrounds. Once they reached a pure genetic background, expression of the transgene was assessed in the mammary gland. RNA was extracted and cDNA generated to analyse the expression of the transgene. The transgene was confirmed to be expressed at the RNA level in the virgin mammary gland (Appendix 1B). Whole-cell protein extracts were prepared from wild-type, virgin transgenic and multiparous transgenic mice and a western blot was performed using an antibody recognizing CDP/Cux (Appendix 1B). Virgin transgenic mice were found to express the transgene at the protein level, however at a much lower extent than the pregnant transgenic mice.

Development of the mammary gland in virgin heterozygous mice

Backcrosses towards the C57BL/6 and FVB/N backgrounds have been performed for all the different lines. Histological and wholemount analyses of mammary glands at 5 weeks, 3 months, 6 months and 12 months have been done, as well as analyses of mammary gland development in pregnant mice. At 5 weeks of age, no difference was noted between mice of the different lines (Appendix 2A). Wholemount analyses show that ductal outgrowth is very similar between the different lines, with ducts having just reached beyond the lymph node. Also, by histology, hematoxylin and eosin (H&E) stainings reveal that no striking difference can be observed between the three lines and the wild-type controls. H&E stainings will be sent to a pathologist to confirm this. Furthermore, I will perform immunohistochemistry to see where and at what levels the transgene is expressed at that stage.

The same analysis was performed on mice at 3 months of age (Appendix 2B). At this time point, we still cannot see differences between p75, p110 and wild-type mice, but we can see a difference in the p200 mice. In the mammary gland of the p200 mouse at 3 months, there is increased branching, with increased number of end buds, suggesting that there is increased proliferation in this gland. Therefore, overexpression of p200 CDP/Cux leads to deregulation of mammary gland development and possibly eventually to tumor development. Immunohistochemistry against the transgene should be done, as well as immunohistochemistry against a proliferation marker, such as PCNA, to confirm my hypothesis. At 6 months and 12 months of age, there was no difference noted between the different lines and the wild-type mice (data not shown).

Development of the mammary gland in pregnant mice
In pregnant mice at day 7.5, no differences were noted between the different lines (Appendix 2C). These sections will be sent to a pathologist to confirm this result.

Development of the mammary gland in homozygous mice
Since the transgene is located on the X chromosome, we suspect that in mice that have only one copy of the transgene on one X chromosome, half of the cells will randomly inactivate the X chromosome containing the transgene. Therefore, generating mice with the transgene located on both X chromosomes (homozygous mice) should resolve that issue and increase transgene expression in the mammary gland. We have generated homozygous mice for the three different lines and found some differences in mammary gland development. Wholemount and histological analyses on heterozygous mice (with one copy of the transgene) versus homozygous mice (with two copies of the transgene) revealed differences in the p75 mammary gland at 5 weeks and the p110 mammary gland at 3 months, suggesting that increasing the expression of the transgene results in an abnormal phenotype (Appendix 3). When comparing wild-type mice with homozygous p75 mice, we can see that in the homozygous mouse at 5 weeks of age, there is a big difference in ductal outgrowth in the mammary gland. Whereas in the wild-type mouse the ducts have just reached the lymph node, in the homozygous mouse, the ducts have already invaded through most of the fat pad (Appendix 3A). When comparing mice at 3 months of age, we can see a difference between homozygous p110 and wild-type (Appendix 3B). We don't see a difference in the p75 homozygous mice at three months. In p110 mice, we can see increased branching and increased number of end buds. Therefore, generating and studying homozygous mice could be a good opportunity to study the role of CDP/Cux in mammary gland development and tumorigenesis.

Expression of the transgene in the mammary gland
By immunohistochemistry, we can see that some cells express the transgene in the virgin normal mammary glands (Appendix 4A). Analysis of transgene expression at the mRNA level in the mammary gland was done for the different mouse lines at 5 weeks and 3 months (Appendix 4B). At 5 weeks, we can detect expression of the transgene in the 3 different lines, however at much higher levels in the p75 homozygotes, which explains why we see a phenotype in these mice. At 3 months, expression is lower than at 5 weeks for the 3 lines, but still detectable. Further analysis will be done to determine expression levels during pregnancy. We expect that transgene expression will be higher in pregnant mice, due to the MMTV promoter that is hormone-responsive.

2-Determine the effect of overexpressing the CDP/Cux p200, p110 and p75 isoforms on mammary gland tumorigenesis in mice having reached a pure genetic background (Task 2)

Transgenic mice overexpressing p75, p110 or p200 CDP/Cux develop mammary gland tumors
While the mice were being backcrossed towards the FVB/N and the C57BL/6 backgrounds, they started to develop tumors. Appendix 5 shows the number of mice that have developed tumors so far: 8 in the p75, 8 in

the p110 and 5 in the p200 lines. These mice developed tumors with long latencies, ranging from 20 to 24 months. None of these tumors metastasized in the primary animal. Tumors of p75 (Appendix 6A), p110 (Appendix 6B) or p200 (Appendix 6C) origin are very heterogeneous. Some are solid carcinomas, others contain areas of squamous metaplasia, or papillary differentiation and others are more glandular. Some tumors contain different histological types within the same mass. These results suggest that CDP/Cux targets malignant transformation in a precursor cell, which can then give rise to different lineages. This suggests that the tumors developed in those mice are basal (*9-13*).

Tumors express the transgene

RNA extracts were prepared from mammary gland tumors and from the adjacent normal mammary gland of the same mouse (Appendix 7A). cDNA was prepared and was used to specifically amplify the transgene. From this analysis, we can see that there is consistently more expression of the transgene in the tumor than in the adjacent mammary gland tissue, with often no detectable level of expression of the transgene in the latter. Western blot was performed to look at the expression of the transgene at the protein level (Appendix 7B). Similarly, we can see that the transgene expression is increased in the tumors versus adjacent tissue. Finally, immunohistochemistry was performed on different tumors using the 1300 antibody, which recognizes all isoforms of the CDP/Cux protein and we can see that the tumors express very high levels of CDP/Cux (Appendix 7C). These results strongly suggest that the tumors are caused by the overexpression of CDP/Cux. However, since the latencies are long, it is probable that CDP/Cux acts in collaboration with other events to promote tumorigenesis.

Tumors are of basal origin

To determine if the tumors are of basal origin, stainings for cytokeratin 6 (CK6) and cytokeratin 14 (CK14) were performed (Appendix 8). CK6 stains progenitor cells and CK14 stains the myoepithelial cells. In the tumors, many areas are positive for CK14, suggesting the presence of many myoepithelial cells (Appendix 8A). In the tumors, most cells are negative for CK6, but some small areas stain positive (Appendix 8B). From these results, we can conclude that we can detect the presence of multiple lineages in the tumors of the transgenic mice and that this suggests that tumors are of basal origin.

These results will need to be confirmed in mice of pure FVB/N background, since this is the background of choice for the study of mammary gland tumorigenesis. Groups of 50 mice have been generated and we are currently compiling the tumors as they develop in these mice. Statistical analyses will be performed on these groups of mice. The study of these tumors will allow us to study metastasis and also targets of CDP/Cux that are deregulated. The β-catenin/wnt pathway will be looked at since mice with targeted deregulation of this pathway develop tumors of similar types to the CDP/Cux tumors (*10*). Other typical players of mammary gland tumorigenesis will also be studied such as Her-2, c-myc and more. Homozygous mice will be used to study mammary gland tumor development in the different lines. Future work could include developing cell lines from the mammary tumors to perform various assays, such as knocking down CDP/Cux and looking at the reduced tumorigenic or metastatic potential of the cells. Also, tumors could be transplanted into nude mice to study the capacity of the tumor cells to metastasize to distant sites in vivo.

3-Study the technique of specific transgenesis used to generate the MMTV transgenic mice through a comparison of the two different CDP/Cux p75 lines generated (Task 4)

The two lines express the transgene at the same locations at similar extent

Finally, I have evaluated the technique of specific transgenesis used to generate the MMTV transgenic mice. To do this, I have compared expression of the transgene in the various mouse lines, specifically the two independent p75 lines, to verify that the transgene is equally expressed in the two lines. This experiment was performed by extracting mRNA from different organs and doing reverse-transcription PCR to determine in what organs and at what levels the transgene is expressed (Appendix 9). Very low levels of expression of the transgene were observed in the brain, liver and mammary gland of the two lines. Comparable medium expression was observed in the lung, heart and kidney. Finally, higher levels of expression were observed in the thymus. In the uterus, there seems to be a difference between the two lines. In line 1, there is high expression of the transgene in the uterus, whereas in line 2, there is no noticeable expression. This difference is possibly due to a difference in the estrous cycle of the female mice since this was not accounted for when doing the experiment. Since the mmtv is hormonally influenced, transgene expression could depend on the estrous cycle. Therefore, for a future experiment, females should be taken at the same day of the cycle. For this part, I had planned to perform in-situ hybridization to study expression of the transgene in the various organs. However, it has proven difficult to design a specific probe to do this experiment. The plan was to design a probe specific for the MMTV-LTR, however, this did not reveal to be optimal since they are many different MMTV-LTRs integrated into the mouse genome, rendering this probe not specific. Therefore, I have performed this study by RT-PCR and I think that it is sufficient to prove that specific transgenesis allows for direct comparisons of independent mouse lines, without any of the problems encountered when doing transgenesis by random integration. The transgenes are expressed at the same level and at the same sites.

KEY RESEARCH ACCOMPLISHMENTS

-CDP/Cux is an oncogene.

-Overexpressing various CDP/Cux isoforms in the mammary gland results in mammary gland tumorigenesis.

-CDP/Cux causes the development of basal tumors, suggesting that it targets the malignant transformation of a precursor cell.

-Developmental anomalies are observed in mice overexpressing various CDP/Cux isoforms.

-Specific transgenesis allows for direct comparison of independent transgenic mouse lines.

REPORTABLE OUTCOMES

Abstracts

-Cadieux, C., Goulet, B., Sansregret, L. and Nepveu, A. *The Role of Short CDP/Cux in Cancer*, Reasons for hope meeting, CBCRA, May 2006. (Appendix 10)

- Cadieux, C., Sansregret, L., Harada, R., Kedinger, V. and Nepveu, A. Short CDP/Cux Isoforms Stimulate Cell Proliferation and Invasion, and Cause Cancer in Mice, Mechanisms and Models of Cancer Meeting, Cold Spring Harbor, August 2006. (Appendix 11)

- Cadieux, C., Goulet, B., Sansregret, L. and Nepveu, A. *The Role of Short CDP/Cux in Cancer* IABCR meeting, October 2006. (Appendix 12)

Oral Presentations

I have presented my work on various occasions to my colleagues:

-February 2006: *The role of Short CDP/Cux Isoforms in Cancer*

-February 2007: *The role of CDP/Cux in Cancer*

-May 2006: Research Advisory Committee Meeting: *CDP/Cux Transgenic Mice: The role of Short CDP/Cux Isoforms in Cancer*

-May 2006: PhD proposal: *The role of Short CDP/Cux Isoforms in Cancer*

CONCLUSION

Through this project I will assess the role of different CDP/Cux isoforms on the development of the mammary gland as well as on tumor development. So far, I have shown that overexpressing p75, p110 or p200 CDP/Cux leads to anomalies in mammary gland development and also leads to tumor formation. I have also shown that CDP/Cux promotes tumorigenesis in a precursor cell since tumors arising in the CDP/Cux mice are very heterogeneous and express cytokines associated with different lineages, namely cytokeratin 6 (precursor cells) and cytokeratin 14 (myoepithelial cells). In the future, I plan to study mammary gland tumor development in pure FVB mice of the different lines, particularly homozygous mice. I also plan to look at putative targets of CDP/Cux to identify which ones are deregulated in tumors. I plan to study, among others, the wnt/β-catenin pathway, since mice in which this pathway is deregulated develop similar tumors. I will also study the metastatic potential of those tumor cells.

"So what section"

My research has identified a new oncogene. I have provided evidence that overexpressing CDP/Cux contributes to the malignant transformation of epithelial cells in the mammary gland and thereby causes cancer in mice. This will allow identification of new targets for therapeutic drug development against breast cancer. Also, in my future work, I plan to identify some targets of CDP/Cux that mediate part of this oncogenic phenotype, which will further help identify more targets for possible therapeutic drugs.

Furthermore, my research has enlightened the fact that overexpressing CDP/Cux seems to be associated with the development of tumors in mice that resemble a specific category of breast tumors called basal tumors in humans. These breast cancers are much harder to treat and often recur because we lack specific molecules to target to kill these cancer cells. My research will possibly help identify one of these targets.

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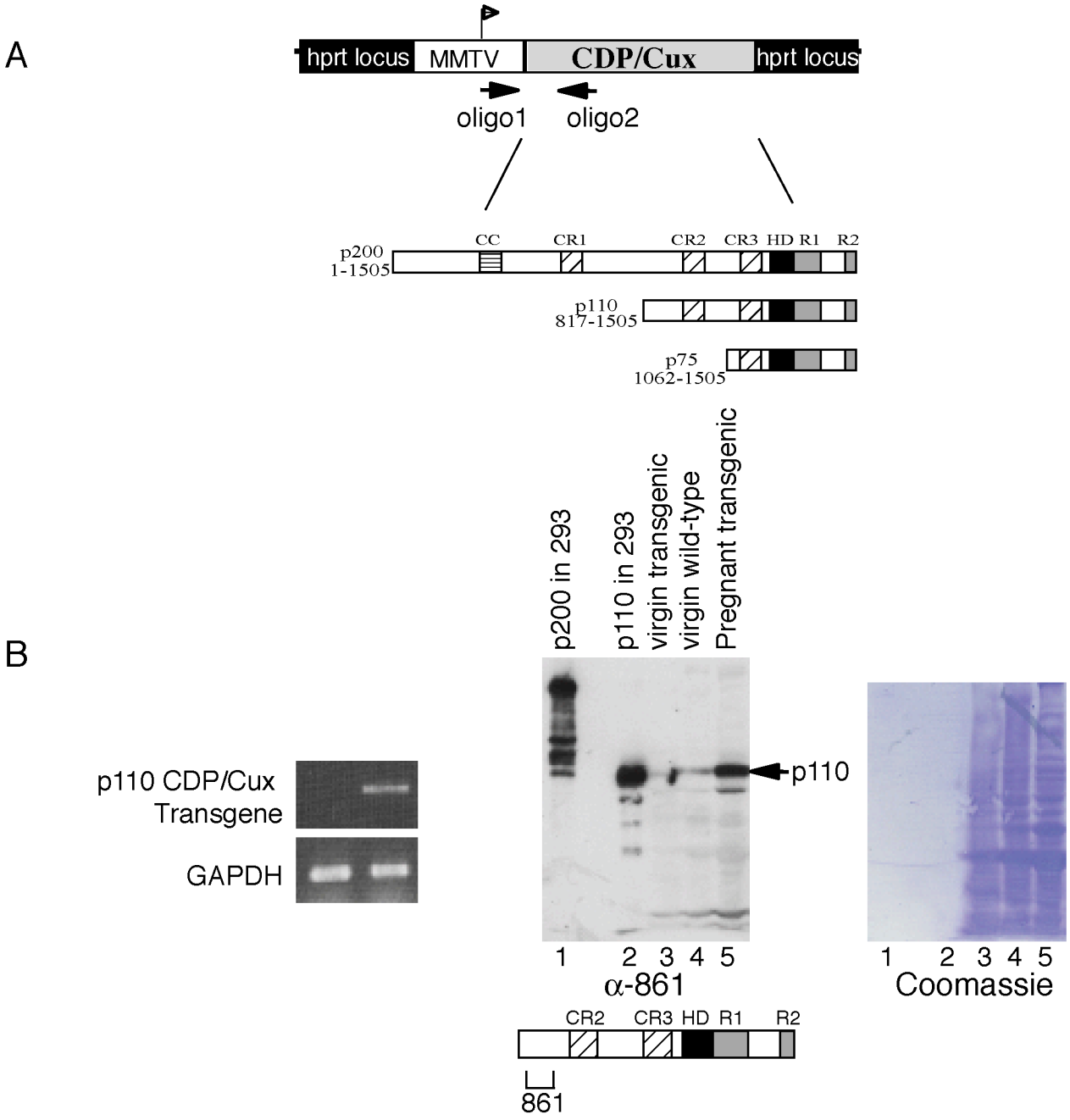
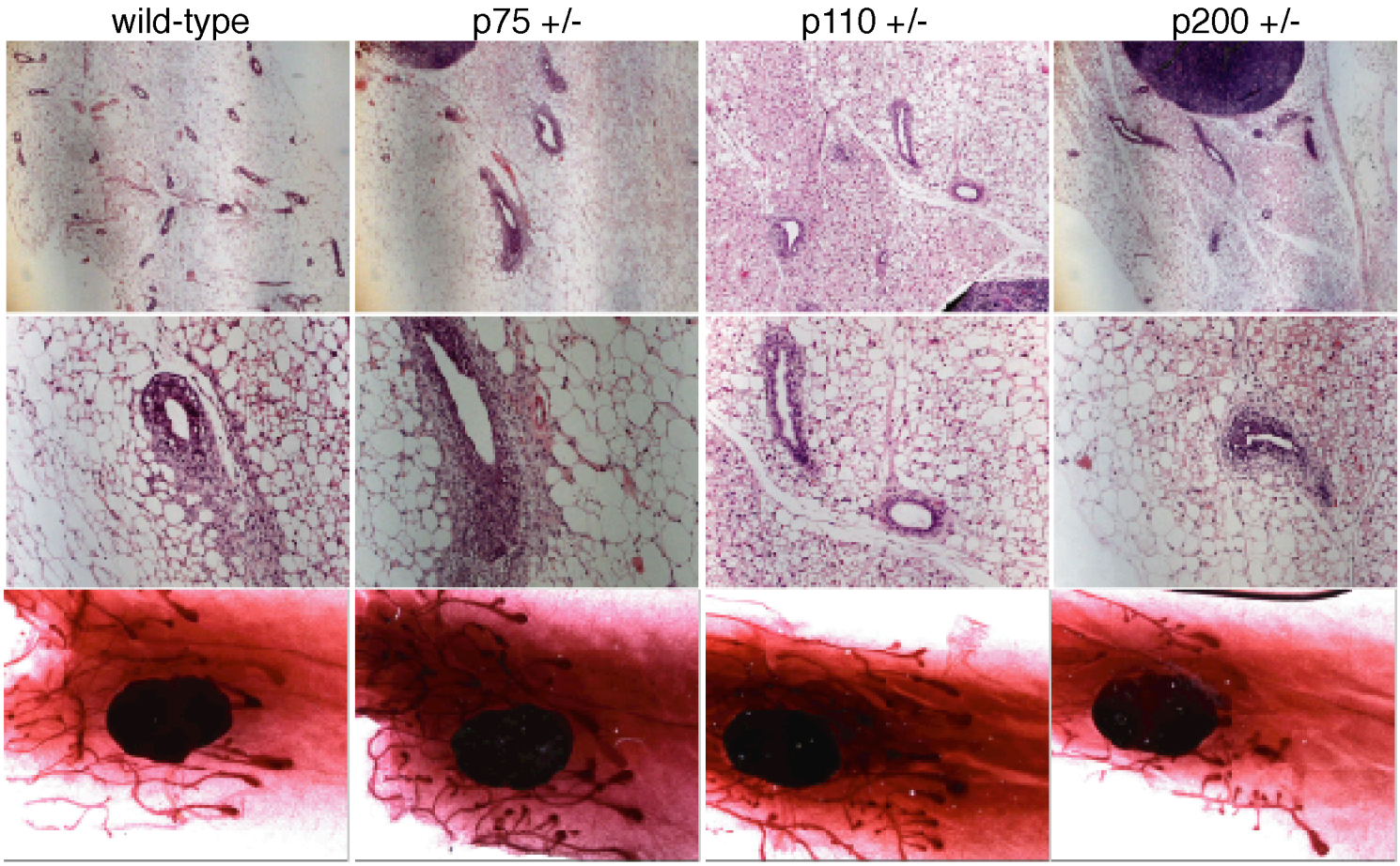
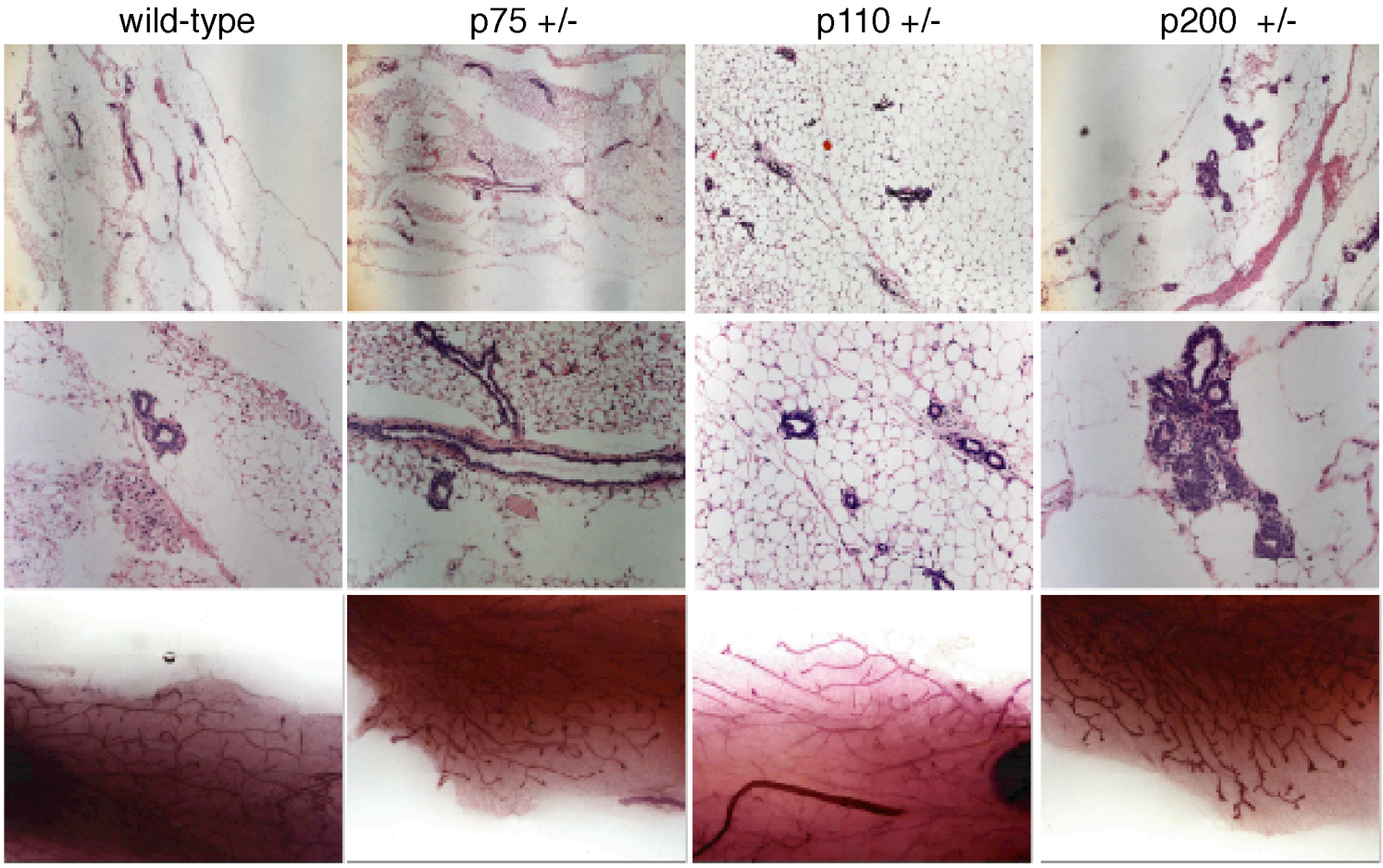
APPENDICES



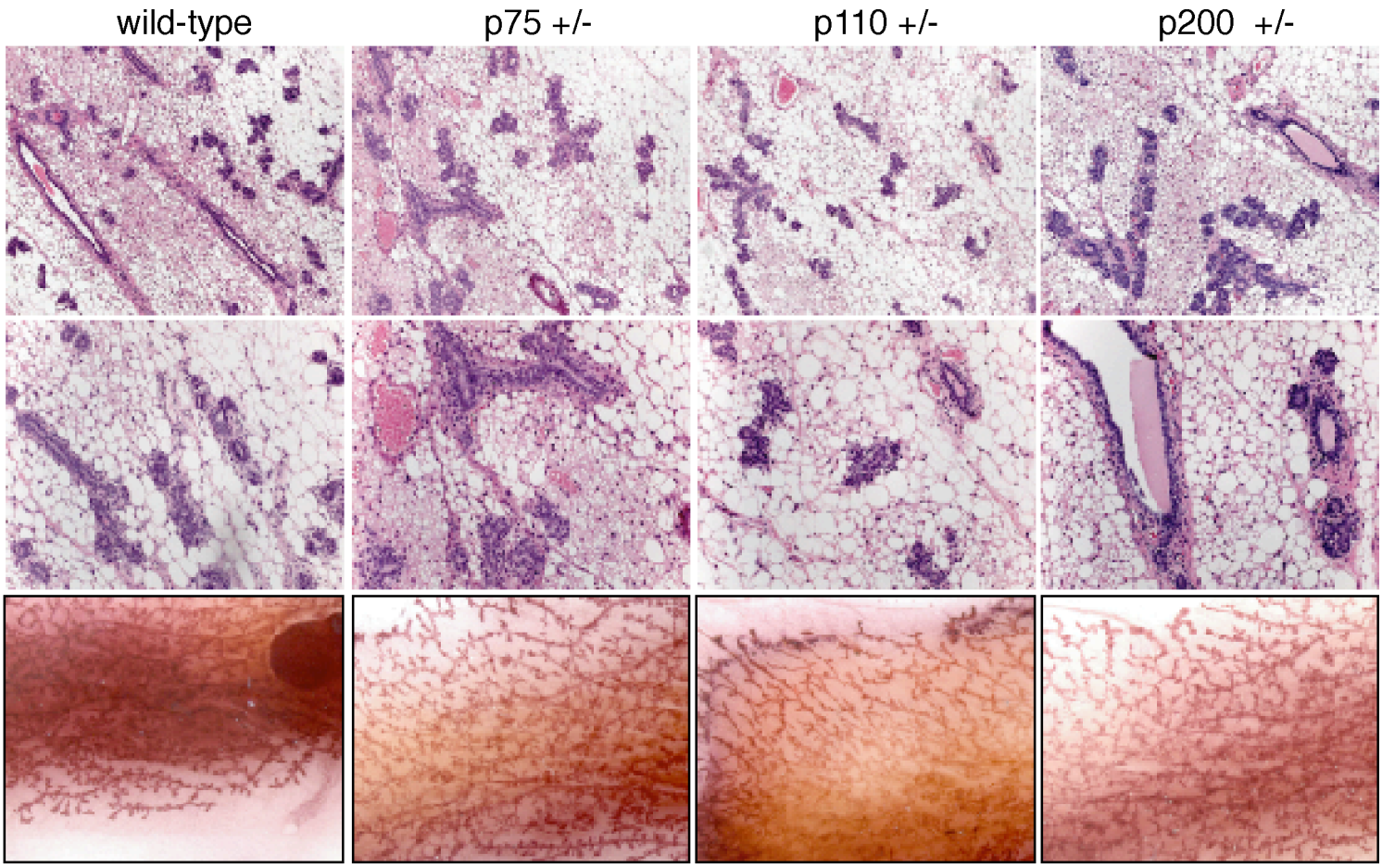
Appendix 1: Transgenic Construct and Expression
(A)The transgene is under the control of the mouse mammary tumor virus promoter and was specifically integrated into the hprt locus. Mice overexpressing either p75 (1062-1505), p110 (817-1505) or p200 (1-1505) CDP/Cux were generated.
(B) The p110 transgene is expressed in the mammary glands of mice both at the RNA level (left pannel) and at the protein level (right panel).



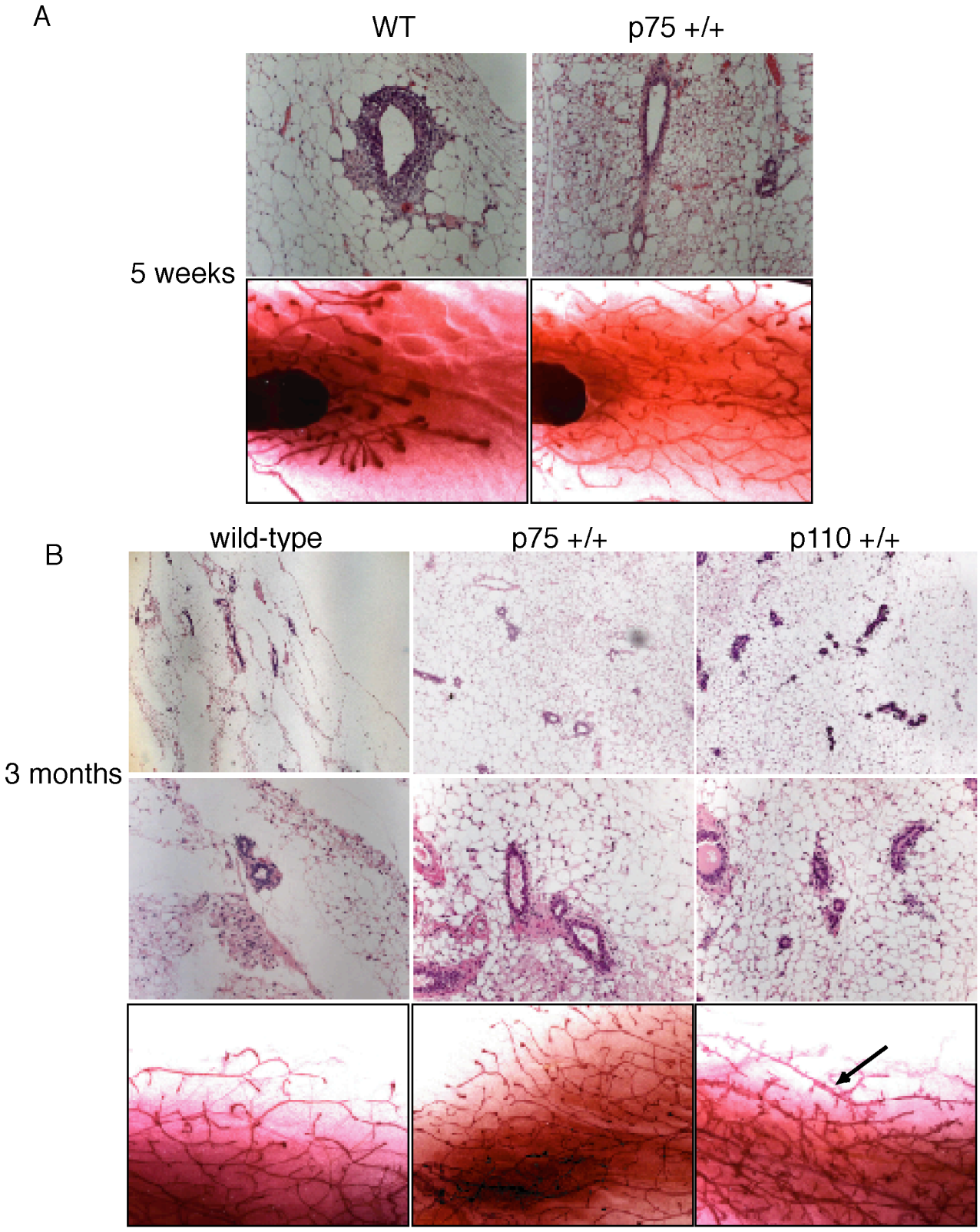
Appendix 2a: Development of the mammary gland in the different transgenic mouse lines at 5 weeks of age
Hematoxylin and eosin stainings on 5υm sections of mammary glands from the different lines were performed. Pictures were taken at orginal magnifications of 100x (top) and 200x (middle). Whole-mounts were performed with mammary glands from each line and stained with hematoxylin to view epithelium (bottom).



Appendix 2b: Development of the mammary gland in the different transgenic mouse lines at 3 months of age
Hematoxylin and eosin stainings on 5um sections of mammary glands from the different lines were performed and pictures were taken at orginal magnifications of 100x (top) and 200x (middle). Whole-mounts were performed with mammary gland from each line and stained with hematoxylin to view epithelium (bottom). Note the increased branching in the mammary gland of p200+/- mice.



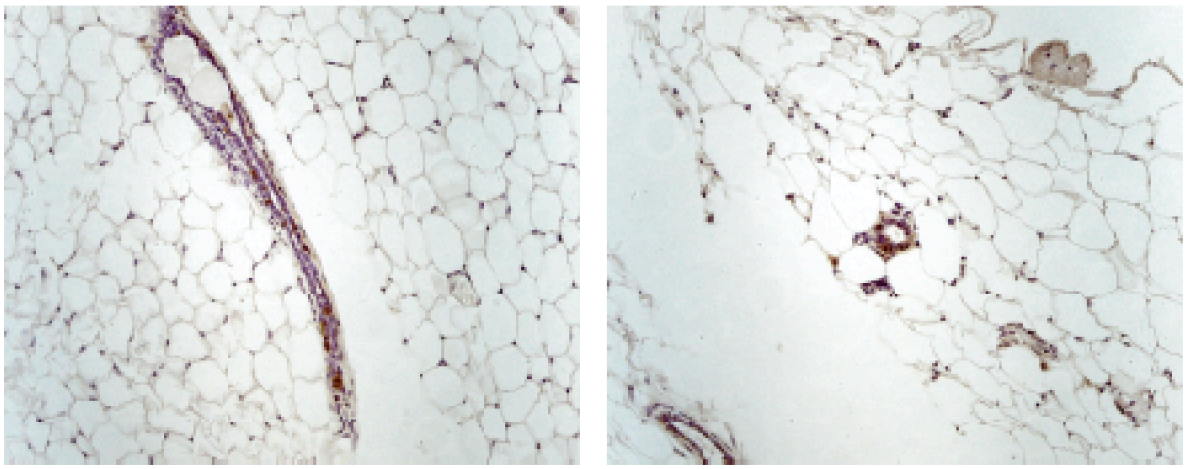
Appendix 2c: Development of the mammary gland in the different transgenic mouse lines at pregnancy day 7.5
Hematoxylin and eosin stainings on 5υm sections of mammary glands from the different lines were performed and pictures were taken at orginal magnifications of 100x (top) and 200x (middle). Whole-mounts were performed with mammary glands from each line and stained with hematoxylin to view epithelium (bottom).



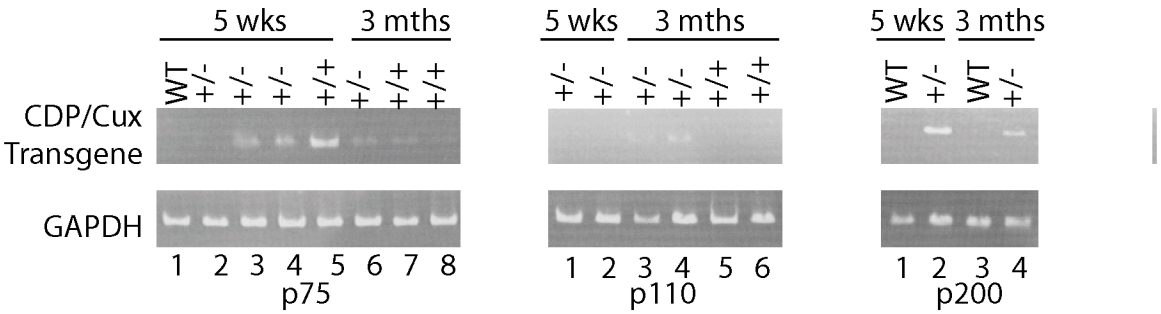
Appendix 3: Development of the mammary gland in homozygous transgenic mice at 5 weeks (A) and 3 months (B) of age

Hematoxylin and eosin stainings on 5υm sections of mammary glands from the different lines were performed and pictures were taken at orginal magnifications of 100x and 200x. Whole-mounts were performed with mammary glands from each line and stained with hematoxylin to view epithelium.

A



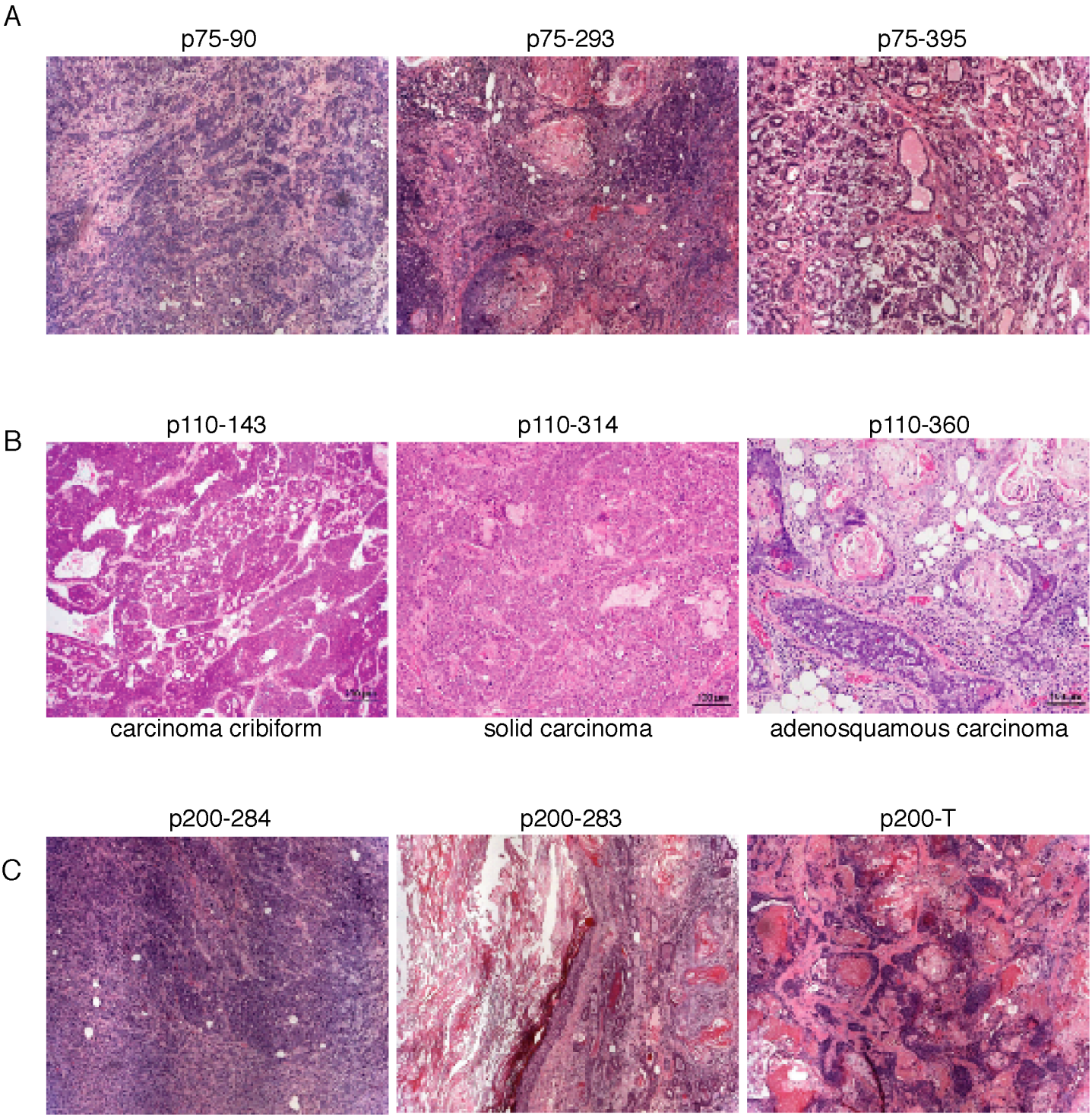
B



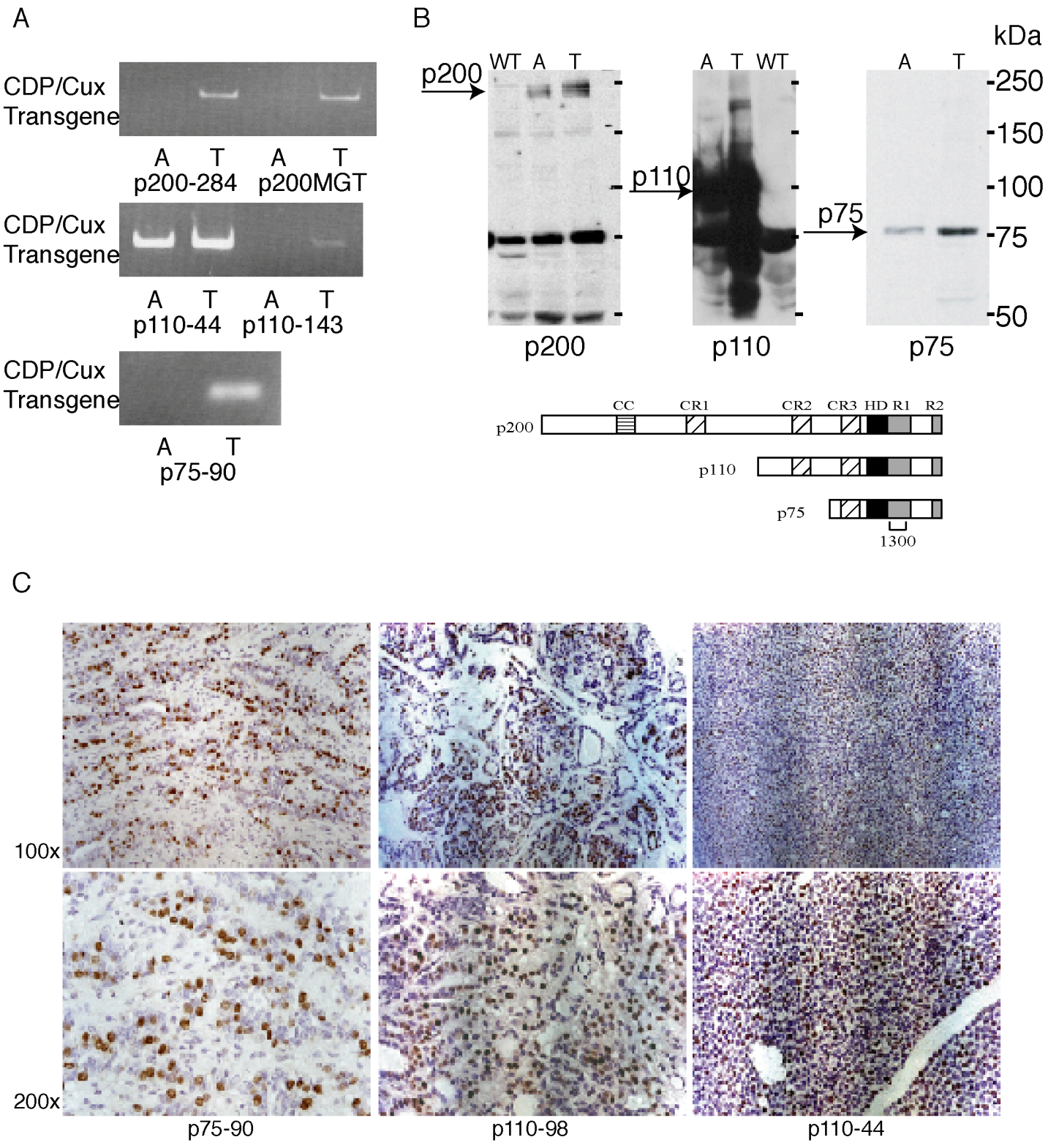
Appendix 4: Expression of the transgene in the developing mammary gland.
(A) Immunohistochemistry using the 1300 antibody. Cells with nuclei stained in brown express the transgene.
(B) Reverse-transcription PCR was done to compare expression of the transgene in mammary glands at different stages of development. Refer to diagram in figure 1 to view the primers used to amplify specifically the transgene.

all	Wild-type	p75	p110	p200
Number of mice with mammary gland tumors	1	8	8	5
Mean latency (months)	30.0	23.3	20.4	24.2

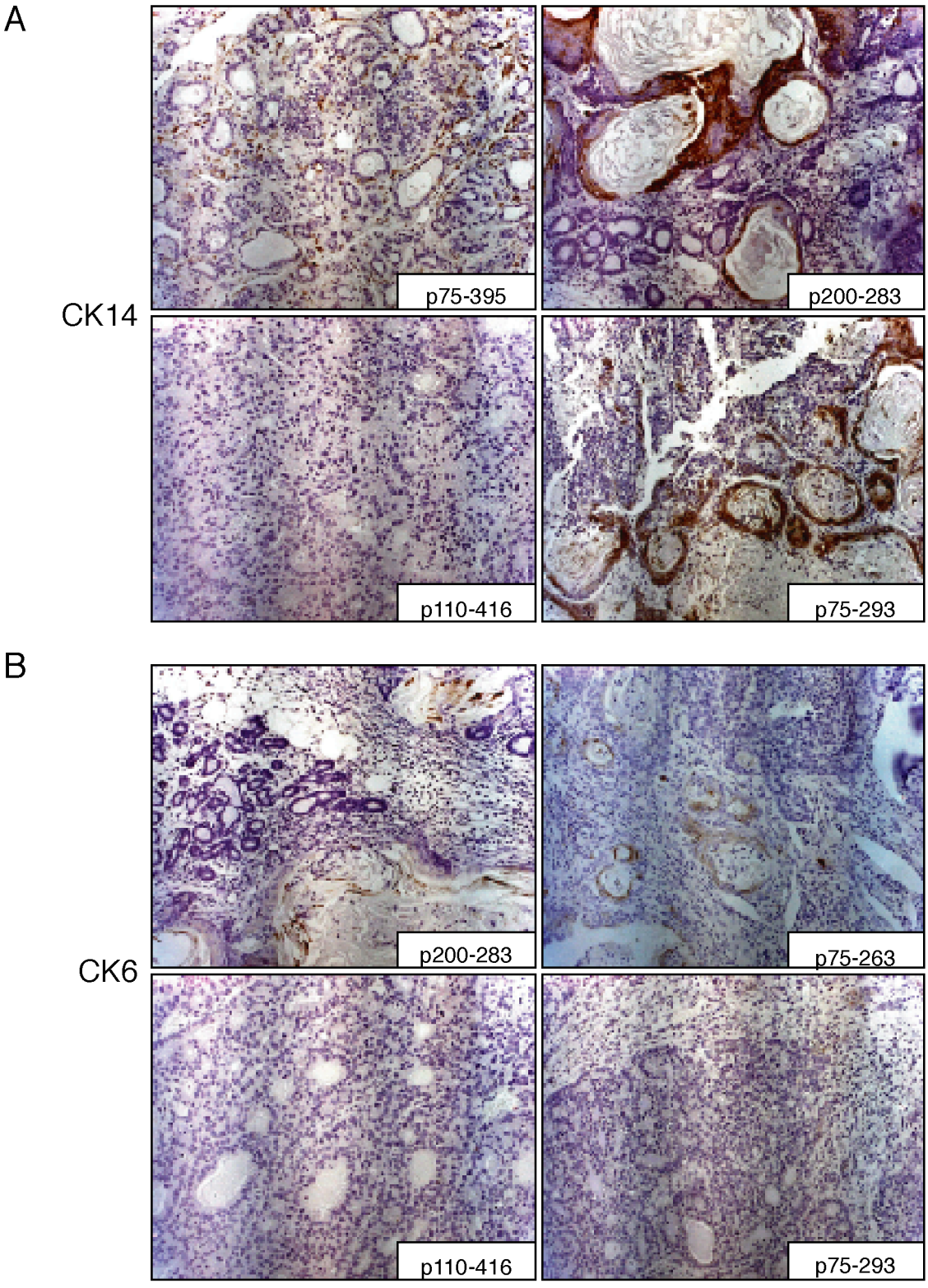
Appendix 5: Number of mammary gland tumors that developed in the different mouse lines of mixed genetic background, as they were being backcrossed towards the FVB/N and the C57BL/6 backgrounds.



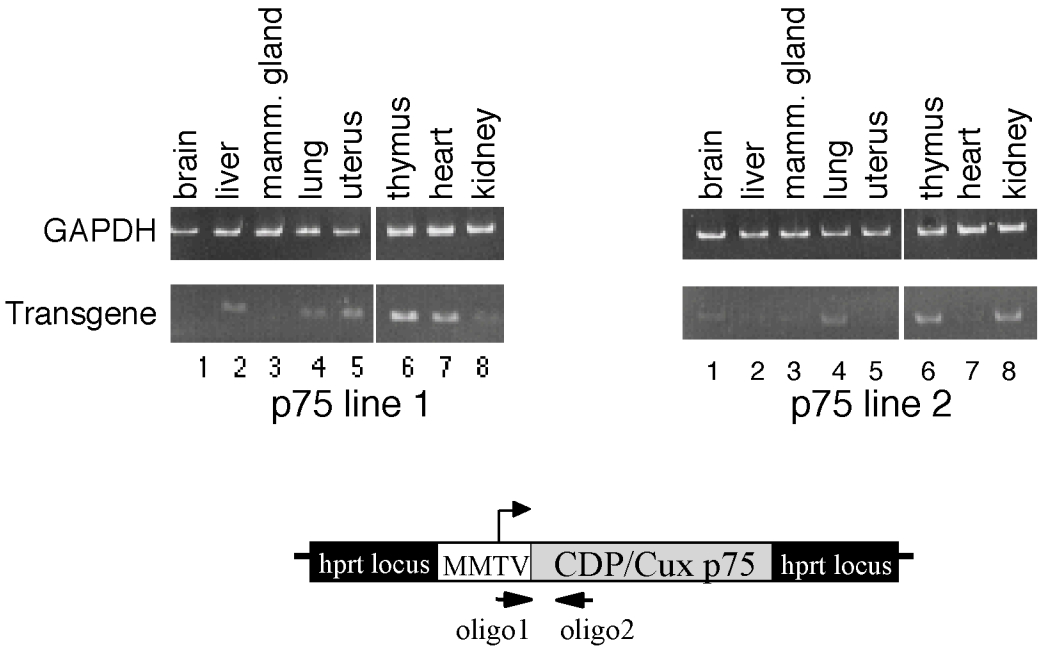
Appendix 6: CDP/Cux transgenic mice develop heterogeneous tumors
Tumors from p75 (A), p110 (B) and p200 (C) CDP/Cux transgenic mice were fixed in formalin and 5υm cuts were stained with hematoxylin and eosin.



Appendix 7: Expression of the transgene in the tumors
CDP/Cux was found to be expressed in the tumors at the mRNA level (A), at the protein level using the 1300 antibody (B) and at the protein level by immunohistochemistry using the same antibody (C). Refer to diagram in figure 1 to view the primers used to specifically amplify the transgene. Abbreviations: T:tumor, A:adjacent mammary gland, Wt:wild-type mammary gland



Appendix 8: Mammary gland tumors are of basal origin.
Immunohistochemistry was performed using antibodies against cytokeratin 6, a marker of progenitor cells, and cytokeratin 14, a marker of myoepithelial cells on tumors from the various transgenic mouse lines.



Appendix 9: The technique of site-specific transgenesis allows transgene expression at similar levels in various organs of independent lines.
mRNA was extracted from various organs of mouse line 1 and mouse line 2. Reverse transcription PCR was performed to amplify GAPDH as a loading control or the transgene specifically.

Appendix 10

Cadieux, C., Goulet, B., Sansregret, L. and Nepveu, A. *The Role of Short CDP/Cux in Cancer*, Reasons for hope meeting, CBCRA, May 2006.

Abstract

The CDP/Cux transcription factor plays a role in cell cycle progression. The full-length protein of 200 kDa is proteolytically processed at the G1/S transition into an isoform of 110 kDa (p110). Short cathepsin L isoforms devoid of a signal peptide were found to be responsible for this processing in the nucleus. A second isoform of 75 kDa (p75) is generated from an mRNA that is initiated within intron 20. In tissue culture, p110 can accelerate cell proliferation by activating the G1/S transition. In addition, the p110 and p75 isoforms are overexpressed in different types of human cancers, such as in leiomyomas and breast cancers. In the present study we investigate the oncogenic potentials of both the p75 and p110 isoforms.

By comparing the expression of CDP/Cux in normal and transformed cell lines we have found that proteolytic processing of CDP/Cux is increased in many transformed cells and was no longer cell cycle regulated. Cysteine protease expression and activity correlated with the extent of CDP/Cux processing. Oncogenic ras caused a rapid increase in transcription and translation of cathepsin L leading to the production of short nuclear cathepsin L isoforms and enhanced processing of CDP/Cux. When ras-transformed 3T3 cells were treated with inhibitors of cysteine proteases, only the cell-permeable compound was able to delay their progression into S phase and their proliferation in soft-agar. Furthermore, over-expression of the processed CDP/Cux isoform was able to stimulate cell motility and invasion.

To further investigate the oncogenic potential of the short CDP/Cux isoforms, we engineered transgenic mice expressing either p75 or p110 under the control of the mouse mammary tumor virus long terminal repeat (MMTV-LTR) by site-specific transgenesis into the hprt locus. We report that 33% of the p75 CDP/Cux mice and 18% of the p110 mice succumbed to a similar disease characterized by hepatosplenomegaly and frequent infiltration of leucocytes into the kidneys and lungs. In most cases, histological and flow cytometry analyses revealed the expansion of a population of mature and immature neutrophils. Some p110 transgenic mice also developed mammary gland tumors.

Overall, these results confirm the oncogenic potential of CDP/Cux p75 and p110, and indicate that ectopic expression of these isoforms can alter the proliferation and differentiation of some hematopoietic and epithelial cells.

Appendix 11

Cadieux, C., Sansregret, L., Harada, R., Kedinger, V. and Nepveu, A.
Short CDP/Cux Isoforms Stimulate Cell Proliferation and Invasion, and Cause Cancer in Mice, Mechanisms and Models of Cancer Meeting, Cold Spring Harbor, August 2006.

Abstract

The p110 and p75 isoforms of the CDP/Cux transcription factor are overexpressed in various types of human cancers, such as in breast cancers and in leiomyomas. The p110 isoform is the product of a proteolytic processing event that normally takes place at the G1/S transition, whereas p75 transcription factor is generated from an alternative mRNA. We used cell-based assays to investigate the cellular functions of CDP/Cux 110 and p75 and we developed mouse models to evaluate their oncogenic potential.

Populations of cells stably expressing p110 CDP/Cux displayed a faster division rate and reached higher saturation density than control cells carrying the empty vector. Constitutive expression of p110 shortened the duration of the G1 phase by 2 to 4 hours. Furthermore, over-expression of the p110 isoform was able to stimulate cell motility and invasion in CDP/Cux KO cells and in NIH3T3 cells. Using ChIP-on-chip analysis, we identified a number CDP/Cux targets involved in cell cycle progression, cell motility and invasiveness.

We engineered transgenic mice expressing either p200, p110 or p75 under the control of the mouse mammary tumor virus long terminal repeat. To compare the oncogenic potential of the 3 isoforms, the transgene was introduced by homologous recombination into the hprt locus of 129/Ola ES cells and, following germ-line passage, was backcrossed onto the FVB and C57BL/6 mouse strains. We report the results obtained with virgin females of backcrosses 1 to 3. 33% of the p75 CDP/Cux mice and 30% of the p110 mice developed a malignancy. While some of the tumors arose from the mammary gland, to our surprise most of the tumors in virgin females originated from various tissues and cell types. Overall, these results confirm the oncogenic potential of CDP/Cux p75 and p110 and reveal a specific spectrum of tumor types for each isoform.

Appendix 12

Cadieux, C., Goulet, B., Sansregret, L. and Nepveu, A. *The Role of Short CDP/Cux in Cancer*, IABCR meeting, October 2006.

Abstract

The CDP/Cux transcription factor plays a role in cell cycle progression. The full-length protein of 200 kDa is proteolytically processed by nuclear cathepsin L at the G1/S transition into an isoform of 110 kDa (p110). A second isoform of 75 kDa (p75) is generated from an alternative mRNA. The p110 and p75 isoforms are overexpressed in different types of cancers, such as in leiomyomas and breast cancers. In tissue culture, p110 can accelerate cell proliferation by activating the G1/S transition. In the present study we investigated the oncogenic potentials of both the p75 and p110 isoforms.

We have found that proteolytic processing of CDP/Cux is increased in many transformed cells and was no longer cell cycle regulated. Cysteine protease expression and activity correlated with the extent of CDP/Cux processing. Oncogenic ras caused an increase in transcription and translation of cathepsin L leading to the production of short nuclear cathepsin L isoforms and enhanced processing of CDP/Cux. A cell-permeable cysteine protease inhibitor was able to delay progression of ras-transformed 3T3 cells into S phase and their proliferation in soft-agar. Furthermore, overexpression of the processed CDP/Cux isoform was able to stimulate cell motility and invasion.

To investigate the oncogenic potential of the short CDP/Cux isoforms, we engineered transgenic mice expressing either p200, p110 or p75 under the control of the mouse mammary tumor virus long terminal repeat, specifically integrated by homologous recombination into the hprt locus. 33% of the p75 CDP/Cux mice and 30% of the p110 mice developed a malignancy. While some of the tumors arose from the mammary gland, most of the tumors in virgin females originated from various tissues and cell types. Overall, these results confirm the oncogenic potential of CDP/Cux p75 and p110 and reveal a specific spectrum of tumor types for each isoform.